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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS BY GAS CHROMATOGRAPHY-MASS SPECTROSCOPY & HPTLC OF *CALOTROPIS PROCERA*

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ABSTRACT

In the present study the major phyto-constituents of leaves hexane extract of *Calotropis procera* investigated qualitatively & quantitatively by GC-MS. A optimize HPTLC profile of leaves extract has developed to resolved and screened the phyto compounds appeared in UV light and after derivatization. In HPTLC analysis seven bands in 254nm, twelve bands in 366nm & five bands after derivatization were observed at different R_f values. Twelve major phyto compounds were identified and estimated by GC-MS analysis. The result obtained by GC-MS study revealed that in hexane extract of leaves, highest peak area % of 25.22 was obtained by Ergost-5-en-3-ol ($C_{28}H_{48}O$) at retention time of 45.020 and the lowest peak area % of 0.24 was obtained by 9 octadecenoic acid 9-Octadecenoic acid (Z)- ($C_{18}H_{34}O_2$) at retention time of 17.353. This study summarizes the information concerning the phyto-constituents present in hexane leaf extract which may be responsible for various therapeutically effects.

Keywords: *C. procera*, Phytochemistry, Hydrocarbons, HPTLC Methods, GC-MS, Metabolites, Leaves.

INTRODUCTION

Calotropis procera (Asclepiadaceae) is a well known Indian medicinal plant & commonly known as Arka [11]. This plant is a perennial herb abundantly found in all part of country (India) and wild in nature. Different parts of this plant used traditionally to cure a number of diseases owing to presence of certain phytochemicals of therapeutic value. It has high

calorific value because of its rich hydrocarbons constituents in leaf. Moreover it was usually applied for tanninizing leather in India. Several attempts have been made in the past to begin it as a biofuel crop [6, 7]. Compounds such as asclepsin and mudarin allegedly isolated from this plant have been found to possess emeto-cathartic, digitalic, bactericidal and vermicidal properties as calotropin is cardio-toxic [8]. Different parts (root, stem, leaves, flowers and seeds) of *Calotropis procera* are traditionally used to cure a number of diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, leprosy and diarrhea [2,7,9,13]. Grazing animals will eat the nearby plant species, but will leave *C. procera* completely undamaged. The animals would come close up to the leaves, smell them and then keep away from the leaves without tasting them. This suggested that neither the toxins nor the sour flavor were causing the grazers to avoid eating the leaves, but quite, there could be a volatile odor or other compound that is acting as a repellant. Assessment between fresh leaves and dried leaves indicated which compounds had evaporated from the leaves and left them palatable to grazers. The dried leaves were treated and analyzed in the same way as the fresh leaves [3]**.** In this research work the plant metabolites present in the leaves (hexane extract) were evaluated qualitatively by applying phyto-chemical tests and quantitatively by gas chromatography-mass spectroscopy (GC-MS) analysis.

Since there is no report available on phyto-components of hexane fraction of *Calotropis procera* leaves extract, it was chosen as the subject of this study. The aim of the present study is to determine the organic compounds in the active fraction of the plant leaves extract with aid of GC-MS. This may provide an insight in its use in traditional medicine.

MATERIAL AND METHOD

Collection of plant material

The leaves of *Calotropis procera* were collected from the plants, grown in IPFT campus, authenticated & identified by Biosciences division of Institute of Pesticide Formulation Technology (IPFT), Gurgaon.

Sample preparation/Extraction

The leaves of the plant were dried in shade to avoid loss of essential oils. Dried leaves were powdered in a mixer grinder and extracted in non polar solvent i.e. by n-hexane in the ratio of 1:5 (one part drug: five part solvent) in soxhlet extractor for 6 hrs maintained at 40^0C. The crude extract was filtered and concentrated in vacuum at 40 ^{0}C using rotary evaporator. The

concentrated extract (hexane) was then dried aseptically with the help of drier and subjected to qualitative phytochemical and GC-MS analysis.

TLC Methodology

1g of *Calotropis procera* leaves was soaked overnight at room temperature in 10ml of n-hexane. The solution was continuously stirred for 6 hrs and kept for next 18 hrs at room temperature & filtered. 10µl of this solution was applied on Merck Aluminum plate pre-coated with silica gel $60F_{254}$ of 0.2mm thickness by linomat IV applicator. The plate was developed in solvent system of Toluene: Ethyl acetate: Formic acid (7.5:2.5:0.5). The plate then air dried and visualized in UV 254 & 366nm. The plate was derivatized in Anisaldehyde- Sulphuric acid reagent and heated at 105 ^{0}C till the colour of the spot appeared on visualization in white light.

Gas chromatography-Mass Spectrum analysis

Analysis of the extract was performed using GC-MSD Shimadzu (Mass Selective Detector, GC-QP 2010 plus MSD model) equipped with DB-5MS fused silica capillary column (Agilent J&W GC column, 5% Phenylated methyl siloxane, 30 m length × 0.25 mm i.d. × 0.25 µm film thickness). GC-MS analysis was carried out using oven programming of initial temperature 50 ^{0}C for 2 min followed by a ramp rate of 20 ^{0}C /min up to a temperature of 130 ^{0}C followed by 12 ^{0} C /min ramp to a temperature of 180 ^{0}C with a hold time of 10 min. The injector temperature was set at 280 ^{0}C in split less mode; solvent delay time was given as 6.5 min. The interface and ion source temperatures were set at 280 ^{0}C and 250 ^{0}C, respectively. MS Quadruple temperature was set at 150 ^{0}C, an emission current of 300 µA, lower vaccume 3.0e+000 Pa and upper vaccume <1.0e-004 Pa. The instrument was operated in Electron Impact Mode (EI) with electron energy 70ev, carrier gas helium was used at a flow rate of 1 mL/min. For confirmation of analytes analysis was done by SIM mode. The compounds were identified by comparing the mass spectral data with those in house library (NIST) already stored in a compact library of chemical substances.

RESULT AND DISCUSSION

TLC Analysis: In TLC profile a number of bands appeared at different R_f under UV 254, 366 & after derivatization. At 254nm seven bands, at 366nm, twelve different bands and 5 bands in white light after derivatization were observed. The details of colour of bands & R_f values are given in **Figure.1 & Table.1**. The TLC study showed that a number of phytochemical

constituents were present in leaves hexane extract of *Calotropis procera.* These may be identify & estimated as mean of spectroscopic technology.

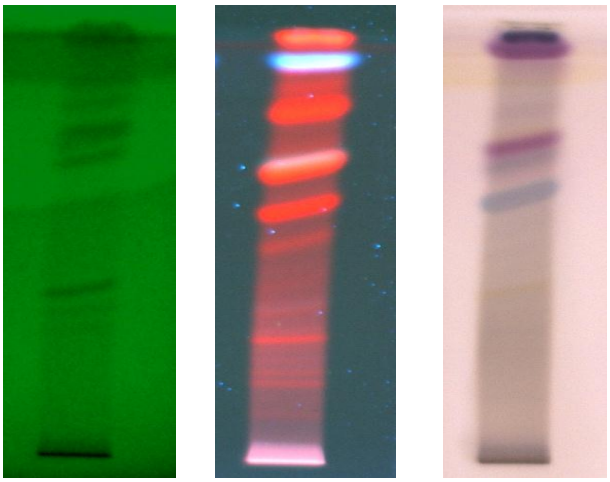


Figure 1: HPTLC fingerprint of leaves *of Calotropis procera*;

Sample, *n*-hexane extract (10µl); Toluene: Ethyl acetate: Formic acid (7.5:2.5:0.5)

Table 1: R_f Values of *Calotropis procera* (leaves) in n-hexane extract.

	Visualization/Detection (R_f Values)		
Samples	**Under UV 254 nm**	**Under UV 366 nm**	**After derivatization**
10 µl of hexane extract	0.40, 0.68, 0.73, 0.78, 0.81, 0.85, 0.97	0.19, 0.21, 0.30, 0.36, 0.43, 0.50, 0.58, 0.68, 0.78, 0.85, 0.91, 0.97	0.62, 0.68, 0.73, 0.91, 0.97

GC-MS analysis of the extract

In the GC-MS analysis of *Calotropis procera*, 12 compounds were identified in n-hexane extract. The identification of chemical constituents is based on the peak area (which represents the percentage of that compound), molecular weight and molecular formula. The chromatogram (Figure 2) of hexane leaf extract shows 12 prominent peaks as Napthalene decahydro2, 6 dimethyl ($C_{12}H_{22}$) with retention time of 7.467 and peak area of 3.16; 2-H Benzofuranone 5,6,7, 7A tetrahydro 4,4,7A trimethyl ($C_{11}H_{16}O_2$) with retention time of 10.88 has the peak area of 0.55; 2-Pentadecanone, 6, 10, 14-Trimethyl ($C_{18}H_{36}O$) with retention time of 14.687 has the peak area of 1.37; Hexadaconic acid, methyl esters ($C_{17}H_{34}O_2$) with retention time of 16.087 has the peak area of 4.79; 9-Octadecenoic acid (Z)- ($C_{18}H_{34}O_2$) with retention time of 17.353 has the peak area of 0.24; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- ($C_{19}H_{32}O_2$) with retention time of 19.662 has the peak area of 0.71; 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]- ($C_{20}H_{40}O$) with retention time of 19.970 has the peak area of 3.99; (6Z), (9Z) Pentadecadien 1-ol ($C_{15}H_{28}O$) with retention

time of 21.142 has the peak area of 11.10; Farnesol isomer ($C_{15}H_{26}O$) with retention time of 36.403 has the peak area of 0.37; Tetratetracontane ($C_{44}H_{90}$) with retention time of 42.462 has the peak area of 2.40; Ergost-5-en-3-ol ($C_{28}H_{48}O$) with retention time of 45.020 has the peak area of 25.22; Stigmasta-5,22-Dien-3-ol ($C_{29}H_{48}O$) with retention time of 45.795 has the peak area of 7.68. The other known peaks at retention times are shown in figure 2. The structures of customary compounds of hexane leaf extract are described in Table 2. The study regards the presence of many secondary metabolites in the leaves of Calotropis procera and provides an overview of different doses of phytoconstituents having led to their biological activities. According to this study the plant extract could be use for treatment of various diseases due to availability of various phyto-constituents responsible for different therapeutical effect. In GC-MS analysis various identified molecules are found to be.

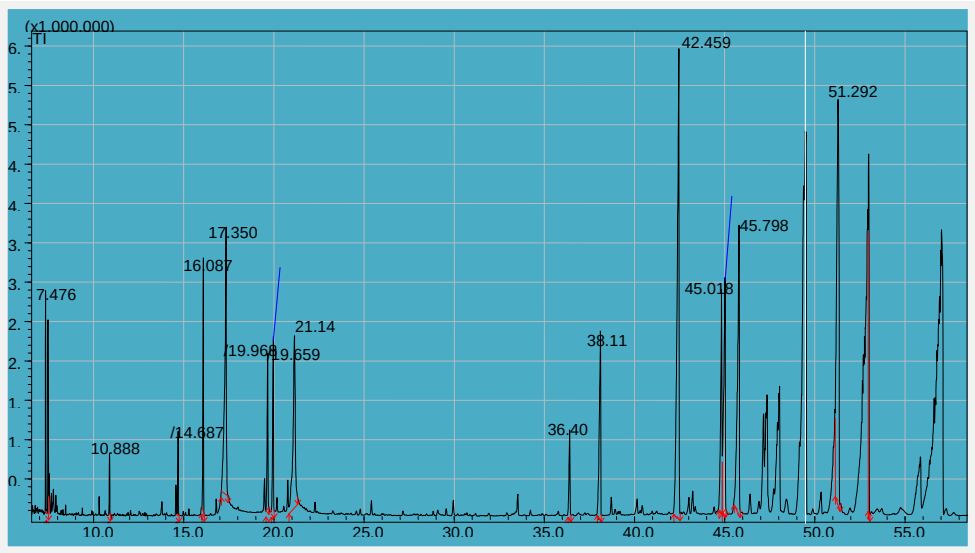


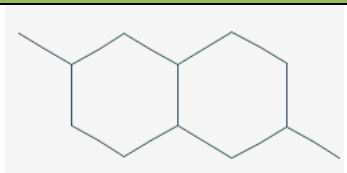
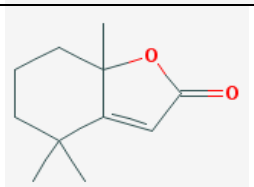
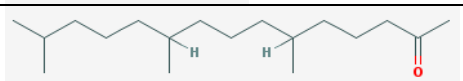
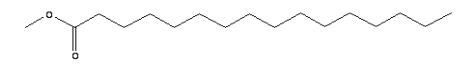
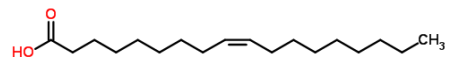
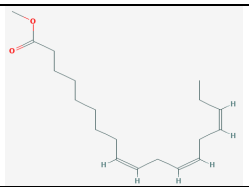
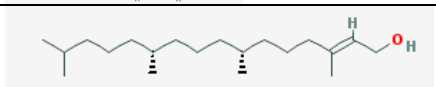
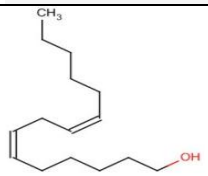
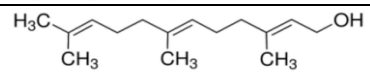
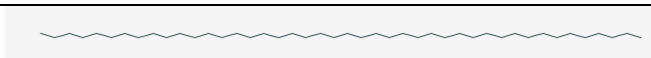
Figure-2 Chromatogram (GC/MS) of the n-hexane extract of *Calotropis procera* (leaves).

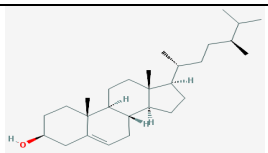
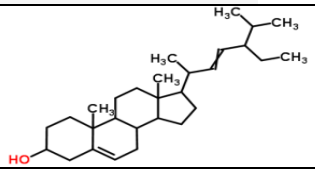
Table2: Chemical constituents present in the hexane extract using GC-MS analysis.

S. No	RT	Fragmentation pattern	Area %	Mol. Wt	Formula	Compound name
1	7.467	81, 55, 95	3.16	166	$C_{12}H_{22}$	Napthalene decahydro2,6 dimethyl
2	10.88	111,67, 37	0.55	180	$C_{11}H_{16}O_2$	2-H Benzofuranone 5,6,7,7A tetrahydro 4,4,7A trimethyl
3	14.687	58,71,55	1.37	268	$C_{18}H_{36}O$	6,10,14-trimethyl Pentadecanone -2
4	16.087	74,87,55	4.79	270	$C_{17}H_{34}O_2$	Hexadaconic acid, methyl esters
5	17.353	73,55,60	0.24	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)-

6	19.662	79,67,55	0.71	292	$C_{19}H_{32}O_2$	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
7	19.970	71, 57,55	3.99	296	$C_{20}H_{40}O$	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]-
8	21.142	67,55,79	11.10	224	$C_{15}H_{28}O$	(6Z), (9 Z) Pentadecadien 1-ol
9	36.403	69,55,93	0.37	222	$C_{15}H_{26}O$	Farnesol isomer
10	42.462	57,71,85	2.40	618	$C_{44}H_{90}$	Tetratetracontane
11	45.020	55,81	25.22	400	$C_{28}H_{48}O$	Ergost-5-en-3-ol
12	45.795	55, 83, 95	7.86	412	$C_{29}H_{48}O$	Stigmasta-5,22-dien-3-ol

Table3: Chemical structure of the most prevailing compounds of hexane extract of *C. procera*

Name of the compound	Chemical structure of the compound
Napthalene decahydro2,6 dimethyl	
2-H Benzofuranone 5,6,7, 7A tetrahydro 4,4,7A trimethyl	
6,10,14-trimethyl, Pentadecanone -2	
Hexadaconic acid, methyl esters	
9-Octadecenoic Acid (Z)-	
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]-	
(6Z), (9 Z) Pentadecadien 1-ol	
Farnesol isomer	
Tetratetracontane	

Ergost-5-en-3-ol	
Stigmasta-5,22-dien-3-ol	

CONCLUSION

The results reveal that the hexane extract of *Calotropis procera* (leaves) have a number of chemical constituents, which may responsible for many therapeutical activities. Further studies are needed to identify, characterize and elucidate the structure of compounds in hexane extract. It is hoped that this study would lead to the establishment of more chemical compounds that could be used to formulate new and more potent drugs of natural origin. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity

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